REMARKS

Rejection of Claims and Traversal Thereof

In the October 6, 2010 Office Action:

1. Claims 1, 5-10, 12-16, 18, 20-22 and 24-27 were rejected under 35 U.S.C. §103(a) as being unpatentable over Lockhart et al., (WO 97/27317), in view of Lakowicz et al., (Photon Spectra (October 2001) 35(10): 96-104 (The Office needs to correctly identify this reference, wherein the lead author is Lakowicz and not Gryczynski,et al.); and in further view of Cao, et al., (Journal of the Amer. Chem. Soc. (July 2001) 123: 7961-7962 and in further view of Qi et al., (Applied and Environmental Microbiology (2001) 67(8): 3720-3727; and

2. Claims 1, 5-10, 12-16, 18, 20-22 and 24-27 were rejected under 35 U.S.C. 103(a) as being unpatentable over Cao, et al., (Nanoparticles within Raman Spectroscopic Fingerprints for DNA and RNA Detection, Science, Aug 2002, Vol. 297, pp 1536-1540, hereinafter Cao); as evidenced by Malicka,et al., (Biopolymers (2003) 72(2) 96-104, hereinafter Malicka) and Lukomska et al., (Biopolymers and Biophysical Research Communication (2005) 328: 78-84) in view of Lakowicz 1 (US Patent Application No. 2002/0160400), and in further view of Lakowicz 2, (Radiative Decay Engineering: Biophysical and Biomedical Applications," Analytical Biochemistry, 2001, Vol. 298, pp 1-24, hereinafter Lakowicz 2).

These rejections are hereby traversed and reconsideration of patentability of the pending claims is therefore requested in light of the following remarks.

Rejections under 35 U.S.C. 103 (a)

Claims 1, 5-10, 12-16, 18, 20-22 and 24-27 were rejected under 35 U.S.C. §103(a) as being unpatentable over Lockhart et al., in view of Lakowicz et al., Cao, et al., and Qi et al. Applicants submit that the proposed combination does not in any way, disclose, teach or suggest the presently claimed invention.

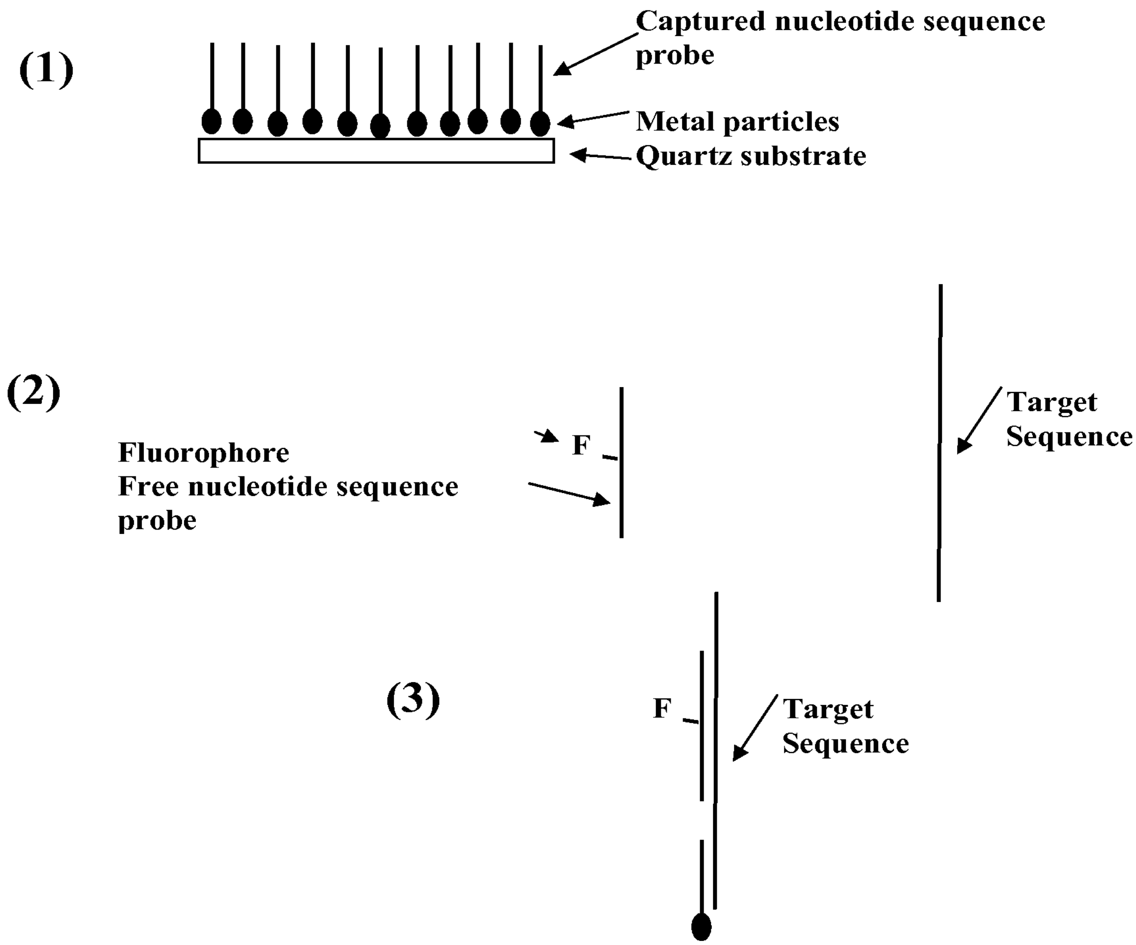
Applicants' claimed invention as recited in claim 1 include:

A method for detecting a *B. anthracis* in a sample wherein a known specific single nucleotide sequence of the *B. anthracis* is the object of detection and a specific first and second section of such a nucleotide is used in the detection method, the method consisting of:

(1) a substrate with immobilized metal particles, wherein the metal particles include a covalently bonded captured nucleotide sequence probe complementary to a first known sequence of a single specific nucleotide sequence of the *B. anthracis* and wherein each of the immobilized capture nucleotide sequence probe **is the same having a specific length and sequence of nucleotides**, and

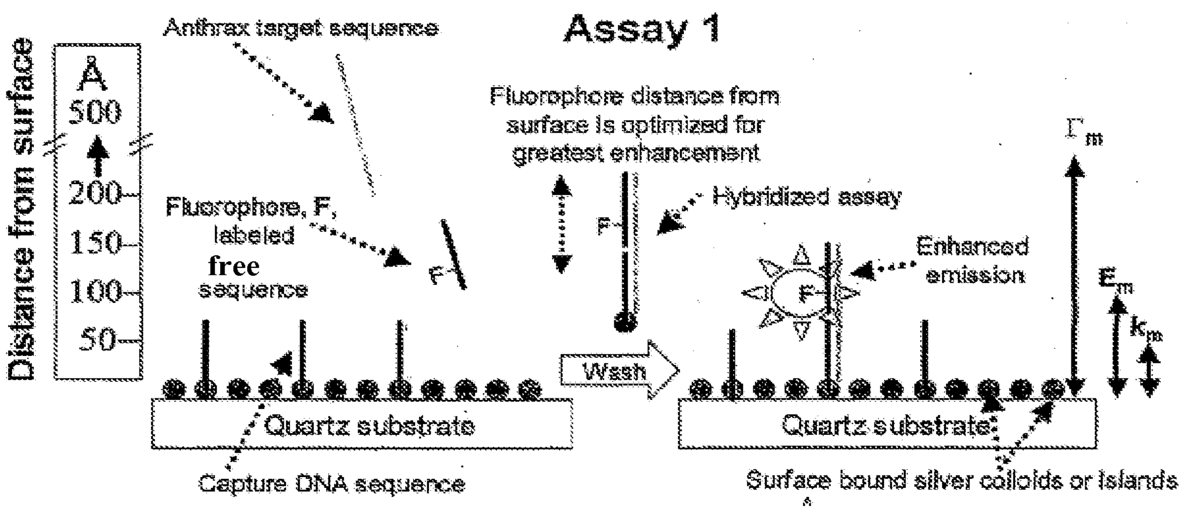
(2) a free nucleotide sequence probe, wherein the free nucleotide sequence probe has been fabricated to a second known sequence of the *B. anthracis* and having an affinity for said nucleotide sequence of *B. anthracis*, wherein each of the free nucleotide sequence probe is the same having a specific length and sequence of nucleotides wherein a fluorophore is attached to the free nucleotide sequence at a specific location and when the free nucleotide sequence hybridizes with the known sequence of the *B. anthracis* the fluorophore is positioned from about 50 to 500Å from the metallic surface,

(3) both of these two probes are necessary to determine if a test sample includes the nucleotide sequence of *B. anthracis*.



It should be very clear that the nucleotide sequence of *B. anthracis* is known so the two probes include sequences that are complementary to only two different regions of the **known anthrax sequence** and are **prepared to attach to only two regions of the *B. anthracis* sequence**. Clearly if there is no *B. anthracis* sequence in the sample then the captured nucleotide sequence probe will remain unbound, and thus, the free probe will not bind to anything and in turn–no signal. In the alternative, if there is *B. anthracis* sequence in the sample then it will attach to the captured nucleotide sequence probe and then the free nucleotide sequence probe will bind to the second site that is complementary to the free nucleotide sequence probe and a signal produced. Thus applicants' system includes the use of two separate and distinct probe sequences (a captured and free nucleotide sequence) that are complementary to only two known different sections of the

target nucleotide sequence. The free nucleotide probe sequence includes a fluorophore positioned a distance from the metal surface. This is shown below by applicant's Figure 1.



Importantly, the use of the two probes that have affinity for only two different nucleotide sequences in the target sequence, under highly stringency conditions, is advantageous because it allows for increased sensitivity. Thus, when both probes are bound to the target sequence there is very little doubt regarding the identity of the bound sequence. In fact using the two probes provides for additional verification that the target sequence is indeed anthrax.

Lockhart teaches a method for identifying differences in nucleic acid abundances. The methods and systems include an array containing a large number (greater than a 1000) of arbitrarily selected different oligonucleotide probes where the sequence of the probe is known and the exact location in the assay tray for placement of such probe is known. Thus, there is a multiplicity of different capture probes. This large number of probes is essential so that differences in the hybridization patterns can be used to determine the differences in the expression of various genes.

Originally the Office made reference to several sections of the Lockhart reference and specifically page 71 and Figures 12 and 13. In this section relating to Figures 12 and 13, the reference describes an array containing a large number (greater than a 1000) of arbitrarily selected different oligonucleotide probes where the sequence of the probe is known and the exact location in the

assay is known. Thus, there is a multiplicity of different capture probes. This large number of probes is essential so that differences in the hybridization patterns can be used to determine the differences in the expression of various genes. Thus each one of the probes is different in Lockhart because they need a multiplicity to deal with all the possible hybridization patterns.

This Lockhart reference provides numerous different types of attached probes to a surface including probes that can include a constant region and a variable region, probes that are complementary to the nucleic acid, probes that are not complementary to the nucleic acid in sample, probes that are random, haphazard, and all possible oligonucleotides of a preselected length, pair of probes wherein each pair differ from each other in preselected nucleotides, etc. It is very clear that Lockhart teaches the use of an array of different probes and states on page 51 that the probes can be random, arbitrary haphazard, composition biased or include all possible oligonucleotides of a particular length. Further on page 53, the reference states that the invention can include 1,000,000 different probes, to provide every probe of a characteristic length that binds to a particular nucleic acid sequence. On page 71, there is a discussion that the probes can include a constant region but if they do they MUST also include a variable region which again provides for the required randomness.

The Office makes further reference to other sections of Lockhart but such sections teach only that all the sequence may have the same number of residues but there is no teaching in this entire Lockhart reference that states that all the probes are the same. **The probes may be the same length but they cannot be the same nucleotide sequence or the Lockhart method would not work.**

Further, regarding labeling of the target nucleic acid in Lockhart, the labeled probes must include a ligatable oligonucleotide and ligase. For determination of the labeled sequence, there must be a ligase involved so that the probe and labeled nucleotide sequence can be ligated together as shown in Figure 12, and recreated below.

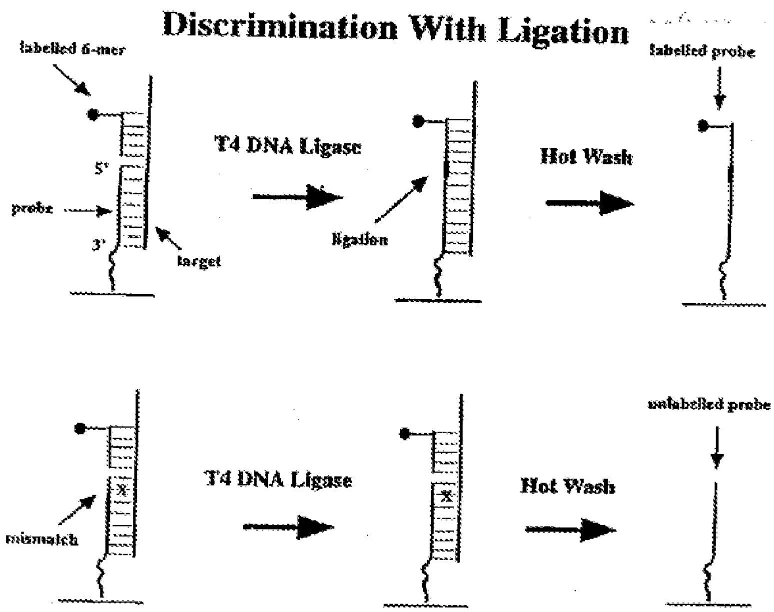


Fig. 12

Thus, the two sequences have to be sufficiently close to allow such ligation and it is very apparent the label must be at the end of 5' end of the ligatable oligonucleotide. Clearly, there is nothing in this reference that discloses or even recognizes the importance of placement of the label for interaction with metallic particles on the substrate.

Still further, Lockhart provides for labels attached to the 5' terminus end of a nucleotide sequence and thus with all the possible lengths of the probes, there is no chance of continuity or the possible placement of a fluorophore at a specific distance from a metallic particle. The present invention demands continuity, that being, a single probe that has affinity for a single sequence area on the target pathogen which will allow the second free probe to attach at the optimal position.

The Office realizes that Lockhart does not provide for any teaching relating to the use of metal particles or metallic surface and does not teach sandwiching a fluorophore between a metal colloid and a metal surface. What the Office has completely overlooked is that the Lockhart also does not teach the placement of a fluorophore at a specific placement on a free nucleotide sequence so that it is positioned at an optimal length from the metallized surface.

According to the Office, Lakowicz teaches a method for increasing the fluorescence of a fluorophore by using metal particles and placement of such fluorophore near such metal. However, the Lakowicz reference does not provide any indication of placement of a fluorophore on a nucleotide sequence that provides any guidance to go in the direction of applicants' claimed invention.

Lakowicz describes a method for determining each nucleotide in a sequence where each individual nucleotide is passed through a tube and the individual nucleotide is surrounded with metal particles, as shown below and recreated for ease of discussion. Notably there are no external fluorophores, only a single nucleotide having intrinsic "glow" ability. It should be noted also that there is no discussion relating to distance of the metallic particles from the single nucleotide for optimal enhancement of "glow" in Lakowicz.

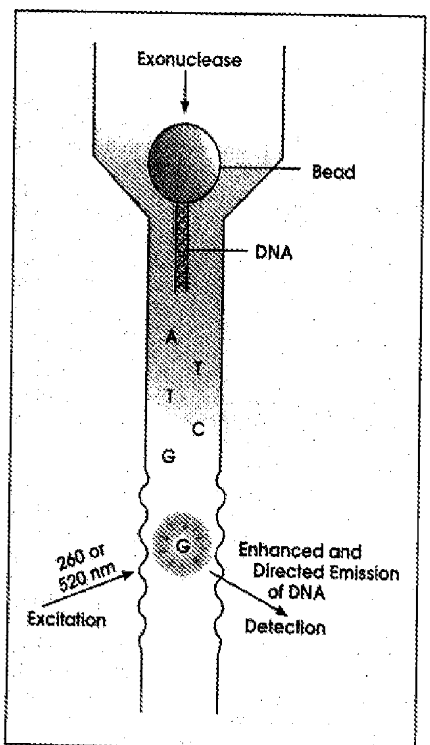


Figure 6. To simplify single-strand DNA sequencing, metal particles could enhance the intensity and provide directionality for emissions of unlabeled nucleotides.

Increased intrinsic emission from DNA may provide new approaches to DNA analysis.

One possibility is single-strand DNA sequencing.[8] The goal is to use exonuclease cleavage of terminal DNA bases that are identified after their sequential release.

A limiting factor in this approach will be the reactive yield of labeling each nucleotide with an extrinsic fluorophore after its release.

Instead, an appropriately designed flow chamber employing metallic particles could enhance the base emission by a combination of the lightning-rod effect, increased radiative rate, decreased lifetime and increased photostability, all contributing to more photons per released base (Figure 6).

The only discussion relating to the addition of an extrinsic fluorophore to the DNA relates to the negative aspects of such extrinsic fluorophores as discussed on page 101 at the bottom of column 1 of the Lakowicz reference, wherein the text expressly states that using extrinsic fluorophores introduces complications including the limiting factor of having to label the DNA. Thus this Lakowicz reference would discourage incorporating labeled nucleotide into the sequence. Thus, it is evident that the Lakowicz reference does not provide any information regarding labeling a DNA nucleotide probe with a fluorophore that is positioned a specific distance from metallic particles.

One skilled in the art would never consider using the teaching of Lakowicz in combination with the Lockhart system because it is very evident that the Lockhart system would no longer operate as intended or it could change the mode of operations. There is **nothing** in Lockhart that would consider the use of **intrinsic fluorophore, that being just the DNA having intrinsic fluorescence.** Clearly, the label of Lockhart is essential to determine if the ligase provided the necessary ligation and if the **label is not there, then the expected hybridization did not occur.**

The MPEP § 2143.01 V – VI states that:

> "If proposed modification would render the prior art invention being modified unsatisfactory for its intended purpose, then there is no suggestion or motivation to make the proposed modification. … [and] If the proposed modification or combination of the prior art would change the principle of operation of the prior art invention being modified, then the teachings of the references are not sufficient to render the claims prima facie obvious."

Further, according to the court in *In re Gordon,* 733 F.2d 900, 221 USPQ 1125 (Fed. Cir. 1984), if proposed modification would render the prior art invention being modified unsatisfactory for its intended purpose, then there is no suggestion or motivation to make the proposed modification and the Office has not established a *prima facie* case of obviousness.

Thus, reduced to the basics, Lockhart teaches a multiplicity of different probes attached to a glass substrate but never mentions the use of metal particle on a surface and never considered placing a fluorophore on a free nucleotide sequence at an optimal distance from the metallic particles. The Lakowicz reference **does not even use a fluorophore** and **in fact discourages the use of same.** Further, there are no probe sequences attached to any metal surfaces. Instead

DNA is passed through a tube that has an inner surface with free metal particles wherein the single nucleotide with some intrinsic fluorescence exhibited intensified fluorescence. In conclusion the combination of Lockhart and Lakowicz does not teach or suggest a detection system comprising a surface substrate with metallic particles and a capture probe sequence attached to the metallic particles, wherein the capture probe can be hybridized to a target sequence. Further this combination does not teach the use of a free sequence probe that once attached to the target sequence positions a fluorophore the optimal distance from the metallic particles to enhance the fluorescence of the fluorophore in a detection method. Finally neither teaches the use of probes wherein all the capture nucleotide probes are the same and all the free nucleotide probes are the same.

The Office has cited several other references in an attempt to establish a *prima facie* case of obviousness, however the addition of Cao or Qi does not rectify the shortcomings of the Lockhart and Lakowicz combination regarding claim 15.

Cao teaches the use of silver nanoballs that are coated with gold to provide the gold plasmonic signals in the use of raman spectroscopy but avoids the downside of silver particles that tend to degrade in DNA hybridization environments. Notably the silver/gold coated nanoballs are free in solution and can be used as a label technique. However, even with this additional piece there is no guidance in any of the references for a detection system comprising a surface substrate with metallic particles and a capture probe sequence attached to the metallic particles, wherein the capture probe can be hybridized to a target sequence. Further this combination does not teach the use of a free sequence probe that once attached to the target sequence (and attached to the capture probe) positions a fluorophore the optimal distance from the metallic particles to enhance the fluorescence of the fluorophore in a detection method.

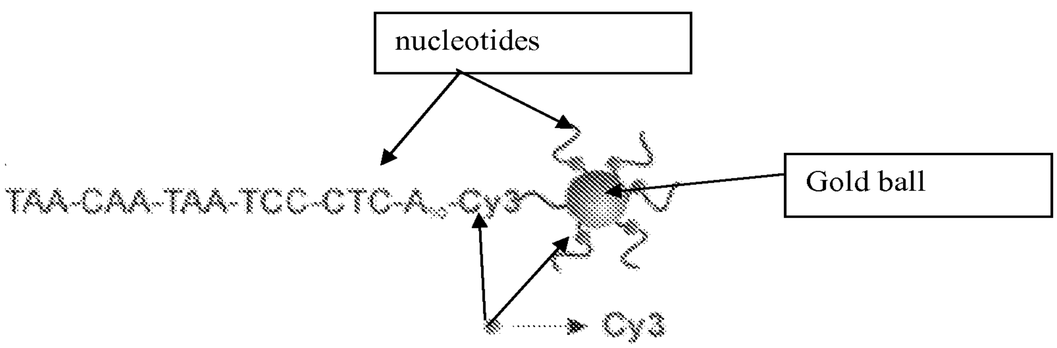
Qi is cited because it is the final piece of the puzzle, that being, the mere mention of Bacillus anthracis. However, if none of the prior art teaches or suggests all the claimed components then the prior art does not defeat the patentability of the pending claims. Applicants request the withdrawal of this rejection under section 103.

2. Claims 1, 5-10, 12-16, 18, 20-22 and 24-27 were rejected under 35 U.S.C. 103(a) as being unpatentable over Cao as evidenced by Malicka and Lukomska in view of Lakowicz 1 and 2. Once

again applicants insist that the proposed combination does not defeat the patentability of the presently claimed invention.

Cao describes the use of gold nanoparticles combined with surface-enhanced Raman scattering spectroscopy for detection and identification of single dye molecules. Cao expressly states that they use this method to overcome the problems related to using molecular fluorophores because of overlapping spectral features and non uniform fluorophore photobleaching rates which could lead to potential complications.

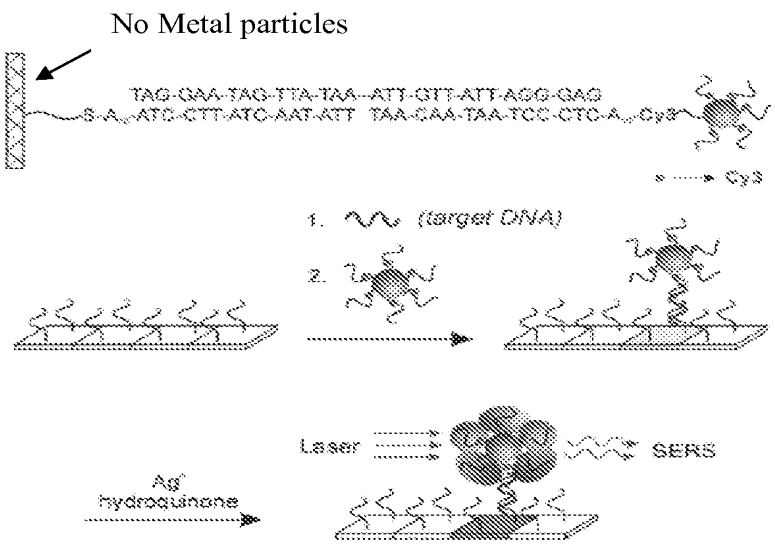
Specifically Cao designed a probe that is built around a 13 nm gold nanoparticle that is functionalized with Raman dye-labeled oligonucleotides. The nanoparticles are coated with a multiplicity of hydrophilic oligonucleotides containing a **<u>Raman dye that is attached to the gold ball.</u>**



After the probe is attached to a small molecule or an antigen it is designed to detect, the substrate is exposed to silver and hydroquinone solution. The silver-plating grows around the Cy3-labeled probes leading to raman scattering occurring in close proximity to the Raman dye, which allows for dye signature detection with a standard Raman microscope.

Notably Cao uses a surface substrate (**<u>such as glass with no metal coating</u>**) that is coated with nucleotide sequences. This surface substrate with the nucleotides attached thereto is kept in **<u>a humidity chamber</u>** at room temperature and then is contacted with oligonucleotide functionalized nanoparticles that include the following (1) Au nanoparticles, (2) a DNA sequence

complimentary to the surface attached nucleotide sequences and also has attached a Cys3 label. Ag containing solution is then added to the assay to increase the size of the metal presence of the Au metal colloid as shown below from Cao:



Notably, this additional of silver ions is attached to the Au particle to grow around the Cy3-labeled Au probe to increase it size to provide for raman scattering. Clearly, the increase in size of the Au particle is of great concern because in fact if the gold particle is too large it has the ability to quench any signal from a fluorophore, so clearly there is no incentive to view the teaching of Cao as credible guidance to go in the direction of applicants' claimed invention

Just by reviewing the figure above from Cao it is very evident that the raman dye is always placed adjacent to the gold nanoparticle. Clearly this reference is not concerned about a specific enhancement positioning of a fluorophore relative to a substrate surface comprising immobilized metallic nanoparticles.

According to the Office, Cao does not teach that immobilized capture probes are immobilized to metal particles on a substrate and that Lakowicz teaches a method for increasing fluorescence intensity of a fluorophore using metal particles. Applicants insist that Lakowicz 1 does not provide any guidance for applicants' invention and certainly does not rectify the shortcomings of Cao.

Relating to the use of DNA or RNA, the Lakowicz 1 reference is limited to the **use of two (2) nucleotides sequences.** One that is attached to a metalized substrate that includes a fluorescence label and then another complementary sequence, as discussed in column 2, page 7, paragraphs [0088] to [0090]. Thus, the probe with fluorescence tag is immobilized on the metallized surface substrate and when another nucleotide sequence is attached to the immobilized probe, the reference states that the fluorescence is enhanced. In another embodiment, the immobilized probe has a donor molecule and a complementary sequence has an acceptor molecule, thus when the hybridization occurs there is a transfer of energy between the donor and acceptor molecule.

Importantly, this reference **never discusses** **the elements of the present invention** with the use of a sandwich assay system as in the present invention. Keeping in mind that the present invention teaches a sandwich assay system in combination with nucleotide sequences wherein a captured nucleotide sequence probe (all capture probes being the same) is attached to a metallized surface substrate and has the ability to capture a target sequence with a known nucleotide sequence. Then a free nucleotide sequence probe (all free probes being the same) can bind to the known sequence, if it has been attached, and the free nucleotide sequence comprises a fluorescence tag that is positioned at the correct placement on the free nucleotide so that when irradiated, the emissions are enhanced because of the correct proximity to the metallized substrate.

Importantly, these two references, that being Cao and Lakowicz 1, teach entirely different methods and concepts and clearly there is no suggestion to modify either reference. The important aspects of Cao include the gold nanoballs attached to the Cys dye and positioned at the opposite end of the substrate. Then the entire setup is bathed in a silver solution to provide a signal. Clearly, looking at the Figures of Cao, the bathing in the silver solution is important and there is no signal without this bathing, as expressly stated by Cao. Notably, the Cao dye label is not bathed in silver until after hybridization with a complementary sequence but the exact opposite is true for Lakowicz wherein the label is attached to a sequence and then hybridization occurs. Further, the metallic particles are in entirely different spaces, the Lakowicz is on the surface of substrate and the Cao is in free space.

Under Graham, and as required by MPEP §§ 2111 and 2141.02, the Office must ascertain the differences between the claimed invention and the prior art, and must consider both the invention and **the prior art as a whole**. Thus, even in light of the *KSR* decision, **the Office must consider**

the inventions of any cited references in their respective entireties. Certain individual features from the references may not be arbitrarily chosen (while equally arbitrarily discarding other disclosed features) to merely lump together disparate features of different references as a mosaic in an attempt to meet the features of the rejected claims. Thus, the Office is not allowed to pick and choose just certain parts of different references and combine them, **but instead, the references in their entirety must be considered.**

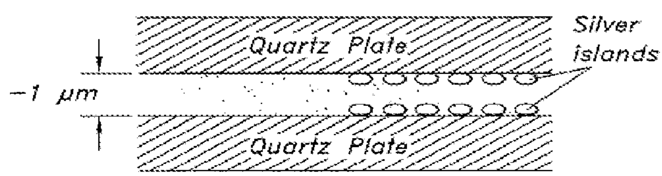
Further, applicant reminds the Office that Section 2143.01 of the MPEP, as well as the ruling in *In re Ratti*, (270 F.2d 810 (CCPA 1959)) state that where a proposed modification or combination would change the **principle of operation of the prior art invention being modified**, then the teachings are not sufficient to establish a *prima facie* case of obviousness. Thus, a combination of references that fundamentally change the "basic principals" under which the prior art was designed to operate cannot support a finding of obviousness. According to the Board in *Ex Parte Vito Cellini*, (Appeal 2008-4104, BPAI 2008), "a change in the basic principles" refers to change that is fundamental in scope so as to **relates to scientific or technical principles of operation.**

Applicants insist that the suggested combination of Cao and Lakowicz will change the "basic scientific principles" of both cited references for the following reasons. Cao describes a critical step that includes the use of silver solution to add to the circumference of the gold nanoball before irradiation. **However, if this silver solution is added to Lakowicz, then the silver particles will increase in size and then with this increase in size, the fluorophore will be positioned to close to the metal particle and the signal will likely be quenched.** If the silver solution is left out of Cao it will not work and certainly will not gain anything by placing a silver coating on the substrate such as Lakowicz because the raman dye needs to be adjacent to the gold nanoball. Further, there are numerous issues relating to use of fluorophore or raman dye because Cao complains about fluorophores. Clearly there is no guidance for picking and choosing from either reference to go in the direction of applicants' claimed invention unless of course the Office is again using the applicants' specification as a blueprint for a hunting expedition, which we all know is considered to be impermissible hindsight.

Thus, the suggested combination of references would require a substantial change in the elements of the prior art as well as a change in the basic scientific principals under which the prior art was designed to operate. As such, the proposed combination does not establish a *prima facie* case of

obviousness because the combination of references fundamentally change the "basic principals" under which the prior art was designed to operate.

Notably, if one combines Lakowicz 2 with Cao and Lakowicz 1, the proposed combination will suffer from the same shortcomings. Lakowicz 2 describes a system that includes two quartz plates with silver islands and DNA was placed between the two plates, as shown below:



Notably, there are no fluorophores used in this Lakowicz system but instead the authors are showing that DNA has the ability to provide intrinsic fluorescence that can be enhanced by metal particles. Notably, there is no discussion in this entire reference relating to optimal placement of a fluorophores or even capturing a target DNA.

Thus, even with the proposed combination there is no teaching or suggestion for all the presently claimed limitations as set forth in the present claims. In light of the foregoing discussion and the fact that all of claimed limitations are not disclose it is clear that the Office has not met its burden of establishing a *prima facie* case of obviousness.

Fees Payable

No fee is due for entry of this response, however, if any additional fee is found due for entry of this amendment, the Commissioner is authorized to charge such fee to Deposit Account No. 13-4365 of Moore & Van Allen.

Conclusion

Applicants have satisfied the requirements for patentability. All pending claims are free of the art and fully comply with the requirements of 35 U.S.C. §112. It therefore is requested that Examiner Bertagna reconsider the patentability of the pending claims in light of the distinguishing remarks

herein, and withdraw all rejections, thereby placing the application in condition for allowance. If any issues remain outstanding incident to the allowance of the application, Examiner Bertagna is requested to contact the undersigned attorney at (919) 286-8089.

Respectfully submitted,

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